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New Bulgarian records of fungi associated with glacial relict plants

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Abstract — The paper contributes to knowledge of Bulgarian fungal diversity on glacial relict vascular plants. Eleven new country records of ascomycetes and anamorphic fungi are presented with descriptions, namely *Asteromella silvarum, Cainiella johansonii, Gnomoniella vagans, Hendersonia culmicola, Leptosphaerulina dryadis, Melasmia mougeotii, Phaeosphaeria nardi, Pseudomassaria islandica, Septoria macropoda, S. tenella,* and *Sydowiella dryadis. Microbotryum silenes-acaulis* is also an addition to the list reflecting the recent changes in *M. violaceum* s. lat. New data are included about the distribution of *Leptosphaeria doliolum, Nodulosphaeria modesta, Ophiognomonia gei-montani,* and *Phaeosphaeria juncina,* all previously recorded in Bulgaria. In addition, a brief review is presented of previously encountered fungal species on glacial relicts in the country.

Key words — taxonomy

Introduction

The study of fungi on relict hosts is of considerable interest as it may contribute to understanding fungal biogeography and fungus-host interactions, given that many parasitic and saprotrophic fungi demonstrate preferences for particular host plants. This research also contributes to better understanding of the need to establish appropriate practices for simultaneous conservation of specialized parasitic and saprotrophic fungi and their hosts (Denchev & Bakalova 2002, Denchev 2005). Research into fungal diversity associated with glacial relict plants has a long history. See Chlebicki (2002) for details and further references on this topic.

Although fungi associated with glacial relict plants have not previously been studied in Bulgaria, earlier taxonomic surveys have revealed a number of ascomycetes, smut, rust, and anamorphic fungi on various Bulgarian relict plants: on *Astragalus alopecurus: Uromyces punctatus* J. Schröt.; on *Carex atrata:*

Puccinia dioicae Magnus; on *Carex curvula: Anthracoidea curvulae* Vánky & Kukkonen and *Schizonella melanogramma* (DC.) J. Schröt.; on *Carex rupestris: Comoclathris deflectens* (P. Karst.) Nograsek and *Physalospora alpestris* Niessl; on *Cerastium eriophorum: Massariosphaeria rubicunda* (Niessl) Crivelli; on *Dianthus microlepis: Microbotryum dianthorum* (Liro) H. Scholz & I. Scholz; on *Dryas octopetala* s. lat.: *Isothea rhytismoides* (Bab. ex Berk.) Fr. and *Wettsteinina dryadis* (Rostr.) Petr.; on *Empetrum nigrum: Duplicaria empetri* (Fr.) Fuckel; on *Gentiana asclepiadea: Cronartium flaccidum* (Alb. & Schwein.) G. Winter; on *Oxyria digyna: Bauhinus vinosus* (Tul. & C. Tul.) R.T. Moore (≡ *Microbotryum vinosum* (Tul. & C. Tul.) Denchev) and *Puccinia oxyriae* Fuckel; on *Poa alpina: Puccinia graminis* Pers.; on *Polygonum viviparum: Bauhinus bistortarum* (DC.) Denchev (≡ *Microbotryum bistortarum* (DC.) Vánky); on *Primula deorum: Uromyces primulae-integrifoliae* (DC.) Niessl; on *Primula minima: Uromyces apiosporus* Hazsl.; on *Rheum rhaponticum: Oidium erysiphoides* Fr.; on *Salix herbacea: Rhytisma salicinum* (Pers.) Fr. and *Melampsora epitea* Thüm.; on *Salix lapponum: Hyaloscypha albohyalina* (P. Karst.) Boud. and *Melampsora lapponum* Lindf.; on *Salix reticulata: Melampsora epitea;* on *Saxifraga stellaris: Puccinia saxifragae* Schltdl.; on *Vaccinium uliginosum: Naohidemyces vacciniorum* (J. Schröt.) Spooner (Hinkova 1960, 1961; Fakirova 1991; Denchev 1994, 1995, 2001; Dimitrova 1995; Denchev & Negrean 2001; Chlebicki 2002; Zwetko et al. 2004).

The glacial refugia in Bulgaria are of undoubted interest, being among the southernmost points in Europe for distribution of a significant number of relict host plants. The authors of this paper aim at further detailed study of the mycota associated with glacial relict plants in Bulgaria. This initial study has focused on some parasitic and saprotrophic fungi on relict vascular plants less sampled during previous mycological studies in this country. The survey has so far produced some interesting new records that are reported below.

Material & methods

Fungi on *Antennaria dioica* (L.) Gaertn. (*Asteraceae*), *Bartsia alpina* L. (*Scrophulariaceae*), *Carex atrata* L. (*Cyperaceae*), *Dryas octopetala* L. (*Rosaceae*), *Festuca pirinica* Horvat ex Markgr.-Dann. (*Poaceae*), *Geum bulgaricum* Pančić (*Rosaceae*), *Juncus trifidus* L. (*Juncaceae*), *Kobresia myosuroides* (Vill.) Fiori (*Cyperaceae*), *Poa alpina* L. (*Poaceae*), and *Silene acaulis* (L.) Jacq. (*Caryophyllaceae*) were examined. Specimens were collected from three major Bulgarian glacial refugia — Vihren area (Pirin Mts), Kozyata Stena area (Stara Planina Mts), and the high mountain zone of Rila Mts (Fig. 1). These were supplemented by examination of older collections of glacial relict plants kept in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOM). All fungal specimens studied have been conserved in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF).

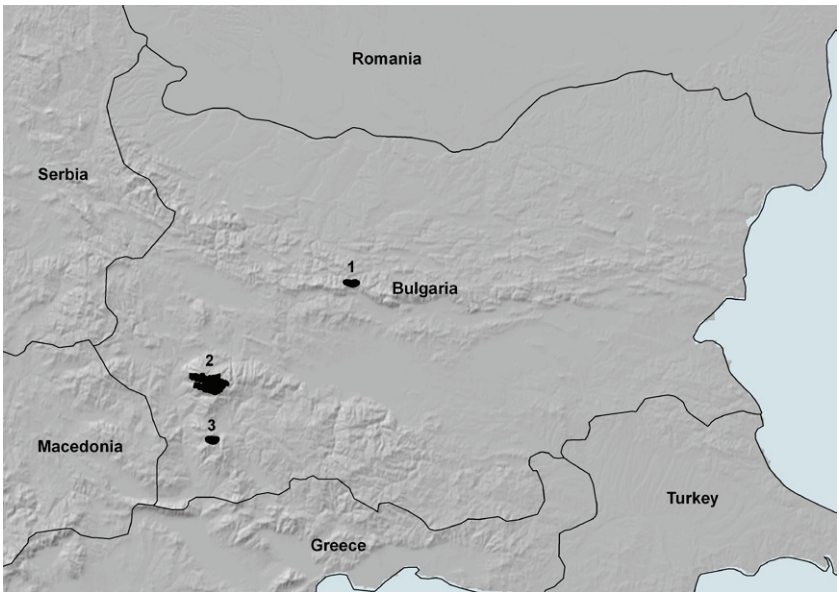

Fig. 1. Collecting areas for fungi on glacial relicts:
1. Kozyata Stena area (Stara Planina Mts), 2. Rila Mts, 3. Vihren area (Pirin Mts).

Microscopic examination was carried out on dried specimens. Slides of ascomata and conidiomata for LM were prepared in lactophenol, the mounting medium being gently heated to boiling point and then cooled. The descriptions below are based on the examined specimens. Primary sources employed for identification of fungi were the works of Holm (1952, 1979), Müller (1957), Barr (1965), Saville (1968), Arx (1970), Vassiljeva (1979, 1987, 1998), Merezhko (1980), Monod (1983), Vanev & van der Aa (1998), and Chlebicki (2002).

Ascomycetes new for Bulgaria

Cainiella johansonii (Rehm) E. Müll., Sydowia 10: 121, 1957['1956'].

Perithecia 100–115 × 170–205 µm, scattered, immersed, with a protruding beak. Beak 650 × 60 µm, long. Asci 137–141 × 37–41 µm, oblong, almost sessile, with refractive apical ring, I–, 8-spored. Ascospores (25–)27.0±1.9 (–32) × (11.5–)13.5±1.6(–19) µm, l/w (1.5–)2.02±0.17(–2.2) (n = 50), almost kidney-shaped, hyaline, finally brown, 1-septate (sometimes constricted at the septum).

Specimens examined — On petioles of *Dryas octopetala:* Stara Planina Mts, Kozyata Stena reserve, 17 Aug 2009, D.Y. Stoykov, B. Assyov & C.M. Denchev (SOMF 29 178); ditto, near the Boba peak, 42°47′05.5″ N, 24°32′45.9″ E, 18 Aug 2009, D.Y. Stoykov, B. Assyov & C.M. Denchev (SOMF 29 173).

Comments — *Cainiella johansonii* was described from Germany (Rehm 1904, as *Lizonia johansonii*) and further reported by Müller (1957) from the Alps (Italy) on *Dryas octopetala.* Later it was recorded in Canadian Arctic on *D. drummondii* Richardson ex Hook. and *D. integrifolia* Vahl (Chlebicki 2002). According to Holm (1979), this fungus is rather common in the Scandes Mountains (Scandinavia).

Gnomoniella vagans Johanson, Öfvers. Kongl. Svenska Vetensk.-Akad. Förh. 41(9): 163, 1884.

Perithecia densely scattered, immersed in leaf tissues. Neck long, protruding. Asci 45–70 × 10–16 µm, clavate, with apical refractive ring, I^-, 8-spored. Ascospores (13.5–)16.2±1.3(–18.5) × (5–)7.2±0.9(–8.5) µm (n = 25), ellipsoid to ovoid, hyaline, non septate.

Specimen examined — On dead leaves of *Dryas octopetala:* Stara Planina Mts, Ushite peak, 13 May 2002, leg. B. Assyov, det. D.Y. Stoykov (SOMF 29 172).

Comments — Barr (1959) considers this fungus to be a rare species.

Leptosphaerulina dryadis (Starbäck) L. Holm, Bot. Not. 132: 86, 1979.

Perithecia small, subpyriform, immersed in leaf tissues. Asci 70–81 × 30–33 µm, very few, saccate, 8-spored. Ascospores 27–34 × 10–12 µm, hyaline, about slipper shaped, upper part rounded, cylindrical below, with 5 transverse septa and one ± incomplete longitudinal septum; several additional transverse and longitudinal septa formed at maturity.

Specimen examined — On dead leaves of *Dryas octopetala:* Stara Planina Mts, Kozyata Stena reserve, near the Boba peak, 42°47′05.5″ N, 24°32′45.9″ E, 18 Aug 2009, D.Y. Stoykov, B. Assyov & C.M. Denchev (SOMF 29 174).

Comments — According to Holm (1979), this fungus is rare. The author suggested that this species is closely related to *Leptosphaerulina pulchra* (G. Winter) M.E. Barr on *Potentilla* spp., the latter distinguished on the basis of less pronounced ascospore septation than in *L. dryadis*.

Phaeosphaeria nardi (Fr.) L. Holm, Symb. Bot. Upsal. 14(3): 124, 1957.

Perithecia single, subepidermal, globose or depressed globose. Asci 62–82 × 9–13 µm, cylindric, 8-spored. Ascospores (19.5–)21.9±3.1(–27.5) × (3–)4.5±0.7(–5.5) µm (n = 50), yellowish to brownish, 7–8-septate; the third (fourth) cell from above swollen, of the same length as the lowest cells and distinctly longer than the second (third) cell; biseriate in the ascus.

Specimen examined — On dry leaves of *Kobresia myosuroides:* Pirin Mts, Kazana, on marble, 1 Oct 1999, leg. P. Vasilev, det. D.Y. Stoykov (SOMF 27 967).

Pseudomassaria islandica (Johanson) M.E. Barr, Mycologia 56: 854, 1965 ['1964'].

Perithecia 190–260 µm in diameter, subglobose, immersed. Setae 200–350 × 17.5–18 µm, dark-brownish. Asci 80–105 × 17.5–22 µm, ±oblong, with

refractive apical ring, I+, 8-spored. Ascospores (21.5–)23.0±1.6(–26.5) × (10.5–)10.7±0.8(–12.5) µm (n = 75), ellipsoid, yellowish-hyaline, apiosporous; irregularly uniseriate in the ascus.

Specimens examined — On dead leaves of *Dryas octopetala:* Pirin Mts, along the track from Vihren peak to Kazana, 2400 m, 41°76′88.7″ N, 23°41′25.2″ E, 11 Aug 2010, leg. I. Apostolova & H. Predashenko, det. D.Y. Stoykov (SOMF 27 631); Rila Mts, Sedemte Ezera, above Babreka lake, 2380 m, 42°12′07.6″ N, 23°18′27.2″ E, 15 Sep 2009, leg. I. Apostolova, det. D.Y. Stoykov (SOMF 27 640).

Sydowiella dryadis Lar.N. Vassiljeva, Mikol. Fitopatol. 13(4): 279, 1979.

Perithecia ca. 400 µm in diameter, superficial, single or in groups, black. Beak 350–700 × 100–120 µm, papillate. Asci 135 × 13 µm, cylindric, 8-spored, with apical ring. Ascospores (14–)16.9±1.7(–19.5) × (6–)7.4±0.62(–9) µm, l/w (1.9–)2.3±0.26(–2.9) (n = 75), ellipsoid, hyaline, two-celled, septum median, slightly constricted at the septa; uniseriate in the ascus.

Specimen examined — On dead twigs of *Dryas octopetala:* Stara Planina Mts, Kozyata Stena reserve, 17 Aug 2009, D.Y. Stoykov, B. Assyov & C.M. Denchev (SOMF 29 177).

Comments — The measurements of asci and ascospores of the Bulgarian specimen fit well those in the original description of the species (Vassiljeva 1979).

Ascomycetes already known from Bulgaria, found on glacial relict plants during the study

Leptosphaeria doliolum (Pers.) Ces. & De Not., Comment. Soc. Crittog. Ital. 1(4): 234, 1863.

Specimen examined — On dry leaves of *Poa alpina:* Stara Planina Mts, Sveti Plast peak, 1820 m, 16 Jul 1952 (ex SOM), fungus comm. & det. D.Y. Stoykov (SOMF 29 175).

Nodulosphaeria modesta (Desm.) Munk ex L. Holm, Symb. Bot. Upsal. 14(3): 80, 1957.

Specimen examined — On dead stems and floral parts of *Antennaria dioica:* Rila Mts, Sedemte Ezera, 7 Jul 2010, D.Y. Stoykov & C.M. Denchev (SOMF 27 629).

Comments — The fungus is so far known in Bulgaria only from Rila Mts on stems and leaves of *Lactuca* (Stoykov 2004).

Ophiognomonia gei-montani (Ranoj.) Sogonov, Stud. Mycol. 62: 58, 2008.

Specimens examined — On dead leaves of *Geum bulgaricum:* Rila Mts, Sedemte Ezera, ca. 2300 m, 21 Jul 1909, leg. B. Davidov (ex SOM), fungus comm. & det. D.Y. Stoykov (SOMF 29 171); Rila Mts, along the track from Smrudlivoto lake to Kirilova Polyana locality, 21 Jul 1998, D.Y. Stoykov (SOMF 22 511); Rila Mts, Yakoroudski circus, on the slopes of Mt Kovatch, above Murtvoto Ezero, ca. 2200 m, 30 Aug 2008, leg. B. Assyov & R. Vassilev, det. D.Y. Stoykov (SOMF 29 179).

Comments — A description of this species based on Bulgarian specimens on *Geum bulgaricum* appears in Stoikov (2000).

Phaeosphaeria juncina (Auersw.) L. Holm, Symb. Bot. Upsal. 14(3): 127, 1957.

Specimen examined — On leaves and floral parts of *Juncus trifidus:* Rila Mts, Sedemte Ezera, in a rock crack, 42°12′97.2″ N, 23°19′21.0″ E, 7 Jul 2010, D.Y. Stoykov & C.M. Denchev (SOMF 27 625).

Comments — In Bulgaria so far recorded only from Vitosha Mt, on *Juncus conglomeratus* L.

Anamorphic fungi new for Bulgaria

Asteromella silvarum Petr., Ann. Mycol. 23: 112, 1925.

Pycnidia epiphyllous, separated, arranged in rows between the veins of the leaves, subepidermal, then erumpent, globose, thick-walled, dark brown, 70–100 µm in diameter. Ostioles central, circular, 8–12 µm in diameter. Conidia rod-shaped, cylindrical, with rounded ends, straight, unicellular, hyaline, 2.5–4 × 1–1.5 µm.

Specimen examined — On leaves of *Carex atrata:* Rila Mts, 2550 m, 24 Sep 1955, leg. I. Bondev (ex SOM), fungus comm. & det. E. Sameva (SOMF 29 164).

Hendersonia culmicola Sacc., Michelia 1(2): 201, 1878.

Pycnidia gregarious between the veins of the leaves, immersed, then erumpent, globose or subglobose, pale brown or cinnamon-brown, 75–115 µm in diameter. Ostioles rounded, 10–15 µm in diameter, surrounded by dark brown cells. Conidia cylindrical, narrow clavate, straight or slightly curved, the base blunt or subtruncate, the apex rounded or pointed, with 3–6 distinct septa, some constricted at the septa, pale olivaceous, in mass brownish, 25–35 × (2.5–) 3–4 µm.

Specimen examined — On dried parts of leaves of *Festuca pirinica:* Pirin Mts, below Vihren peak (2900 m), 9 Aug 1938, leg. B. Achtarov (ex SOM), fungus comm. & det. E. Sameva (SOMF 29 165).

Melasmia mougeotii (Desm.) Arx, Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2de Reeks 51(3): 106, 1957.

Leaf spots black and spread out on both surfaces of leaves. Stromata amphigenous, mostly hypophyllous, crustaceous, flat, subepidermal, black, gregarious, discrete, sometimes confluent in clusters, multilocular; circular, 1–2 mm in diameter, or oblong and irregular, 3–5 × 1–2 mm. Loculi rounded, 125–250 µm in diameter. Conidiophores filiform, straight, hyaline, 20–30 × 1 µm. Conidia numerous, cylindrical with rounded ends, straight or slightly curved, unicellular, hyaline, (3.5–)4–6(–7) × 1.5–2(–2.5) µm.

Specimens examined — On leaves of *Bartsia alpina:* Pirin Mts, below Vihren peak, 2009, leg. V. Vladimirov, det. E. Sameva (SOMF 29 168); ditto, 11 Aug 2010, leg. H. Pedashenko & I. Apostolova, det. E. Sameva (SOMF 29 169).

Septoria macropoda Pass., Fungi Parm. Septor.: no. 141, 1879.

Pycnidia mainly epiphyllous, scattered or arranged in irregular lines, globose or slightly flattened, semi-imersed, thin-walled, brown, 55–125 µm in

diameter. Ostioles rounded or somewhat ellipsoidal, 15–25 μm in diameter. Conidia filiform, straight or curved, mostly with obtuse ends, some slightly tapered at the apex, unicellular or with 2–3 indistinct septa, hyaline, 20–40 × 1–1.5 μm.

Specimen examined — On leaves of *Poa alpina:* Rila Mts, Skakavitsa, 20 Aug 1956, leg. S. Petrov (ex SOM), fungus comm. & det. E. Sameva (SOMF 29 166).

Septoria tenella Cooke & Ellis, Grevillea 8(45): 11, 1879.

Pycnidia spread over both leaf surfaces, gregarious or forming indistinct rows, globose or subglobose, immersed, thick-walled, dark brown, 120–220 μm in diameter. Ostioles indistinct. Conidia filiform, straight, curved to arch-shaped or slightly flexuous, gradually tapered towards the both ends, subacute at the apex, unicellular, hyaline, 22–100 (–109) × 1–1.5 (–2) μm.

Specimen examined — On leaves of *Festuca pirinica:* Pirin Mts, below Vihren peak, 2900 m, 9 Aug 1938, leg. B. Achtarov (ex SOM), fungus comm. & det. E. Sameva (SOMF 29 165).

A smut fungus new for Bulgaria

Microbotryum silenes-acaulis M. Lutz et al., Mycol. Res. 112: 1289, 2008.

An anthericolous smut fungus on *Silene acaulis* has been reported from Bulgaria by Klika (1926) and Denchev (2001), as a species of *Ustilago* or *Microbotryum,* respectively. In 2008, a new cryptic species of *Microbotryum* on *Silene acaulis* was described as *M. silenes-acaulis.* Most probably, the following records refer to this cryptic species and therefore, it is considered here as a new species for Bulgaria.

Literature records — On *Silene acaulis:* Rila Mts, below Mussala peak, ca. 2900 m, J. Klika (Klika 1926, as *Ustilago violacea;* Denchev 2001, as *Microbotryum violaceum* s. lat.).

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